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A COMPARISON BETWEEN FIBRINOLYSIS OF FRESH AND AGED CLOTS OR THROMBI BY rt-PA IN VIVO, EX VIVO AND IN VITRO U. Nauland, W. Haarmann, T.H. Müller, W.G. Eisert. Department of Biological Research, Dr. Karl Thomae GmbH, 7950 Biberach, Federal Republic of Germany

In view of the therapeutic applications of rt-PA it is of interest to investigate whether there is any difference in the lysability between fresh and aged thrombi. The efficiency of fibrinolysis by rt-PA was studied in 3 different ways: In vivo, by measuring the thrombus weight of fresh (1 h) or aged (24 h) thrombi in the carotid artery of rabbits which had been treated with rt-PA (0.4 mg/kg) or saline for 1 h. Ex vivo, by measuring I^{125}-release of in vivo fresh (1 h) and aged (24 h) thrombi (labelled with I^{125}-fibrinogen) suspended in vitro in plasma containing rt-PA (1 µg/ml); the thrombi were formed in the jugular vein and the carotid artery of each rabbit. In vitro, by measuring I^{125}-release of fresh (1 h) and aged (6 or 24 h) human native whole blood clots, PPP-clots, PRP-clots and squeezed PPP-clots which were formed and lysed in vitro with rt-PA (1 µg/ml). In vivo as well as ex vivo rt-PA lysed fresh (1 h) thrombi much better than aged (24 h) thrombi. This difference was more pronounced immediately after the onset of fibrinolysis, but decreased with time. However, in vitro relatively little difference was observed in fibrinolysis efficiency between fresh (1 h) and aged (6 or 24 h) clots; fibrinolysis of these clots was decreased (PPP > whole blood > PRP) with increasing clot retraction, which was almost complete within 1 h. This result was also confirmed when PPP-clots were "retracted" by simply squeezing them just before lysis. Therefore we conclude that a considerable difference in lysis efficiency between fresh and aged thrombi was only observed when thrombi were formed and aged in vivo. This difference was less pronounced with increasing fibrinolysis time.

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MYELOPROLIFERATIVE DISEASE CHARACTERIZED BY THROMBOSIS, BLEEDING AND PLATELET DYSFUNCTION IN MICE INJECTED WITH THE POLYOMA MURINE LEUKEMIA VIRUS (PyMLV). G. Di Minno(1), A. Fusco(2), G. Portella (3), A.M. Cerbone(1), C. Iride(1), G. Tajana(1), O. Russo(1) and P.L. Mattioli. Depts of Experimental Medicine and Anatomy, University of Reggio Calabria at Catanzaro(1); CNR Dept of Experimental Endocrinology and Oncology(2) and Dept of Molecular and Cellular Biology and Pathology, Naples University(3), Italy.

After i.p. injection of PyMLV, NIH/OLAC mice showed thrombi in tail veins, ears, muscles and mesenterium together with thrombi and hematomas of subcutaneous tissues. This was followed by infarctions of lungs, brain and heart, that caused death of the animals. Laboratory evaluations of the infected mice showed normochromic anemia, mild thrombocytosis and marked defects in the aggregation and in the secretion of ATP from platelets exposed to ADP, collagen, thrombin or A23187. About 10% of cells present in the bone marrow was formed by blasts; 20% by multinucleated cells identified as megakariocytes (M) by peroxidase and acethylcholinesterase staining, and the vast majority of the other cells by entities belonging to all stages of maturation of the myeloid lineage. Hybridization experiments showed that the blasts present in the bone marrow were the only cells in which viral replication takes place. Maturation of M in the bone marrow was completely normal, and as for M from non-infected mice, proliferation and maturation in vitro was dependent on the presence of interleukin 3. Finally, studies in other strains of mice showed that the Fv-2 locus is involved in the pathogenicity of PyMLV in NIH/OLAC mice. We conclude that, in addition to its obvious pathophysiological significance, the myeloproliferative disease that occurs in mice after i.p. injection of PyMLV, can serve as an important probe for understanding basic events leading to bleeding and thrombosis.

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THE THIENOPYRIDINE PCR 4099 INHIBITS THE ADP AGGREGATION PATHWAY OF HUMAN PLATELETS BY INTERFERING WITH THE BINDING OF FIBRINOGEN TO THE GLYCOPROTEIN IIb-IIIa COMPLEX. C. Gachet (1), A. Stierlé (1), C. Bouloux (2), J.-P. Maffrand (2) and J.-P. Cazenave (1). INSERM U.311, Centre Régional de Transfusion Sanguine, Strasbourg, France (1) and Sanofi Recherche, Toulouse, France (2).

The thienopyridine, PCR 4099, is a synthetic structural analog of ticlopidine. After oral administration in man, it prolongs the bleeding time (BT) and inhibits ADP-induced aggregation. The aim of the study was to evaluate the effects of oral administration of 200 mg per day PCR 4099 to 10 human volunteers for 7 days on primary hemostasis and to study the mechanism of inhibition of the drug on the ADP-fibrinogen-GPIIb-IIIa pathway of aggregation. BT (measured by a Simplate device) was 4-8 min before treatment and 30 min after 7 days of treatment. Platelets were washed and resuspended in Tyrode's buffer containing apyrase and 0.35 % human albumin. Washed platelet suspensions were used at 37°C for aggregation and fibrinogen binding studies. Human fibrinogen was purified by successive ether precipitation and gelatin affinity chromatography to remove fibronectin. Fibrinogen was pure by SDS-PAGE and > 95 % clottable by thrombin. It was labeled with ^{125}I by the Iodogen method. The binding of ^{125}I-fibrinogen to intact washed platelets exposed to ADP or thrombin was measured after centrifugation at 11,000 g for 1 min in the presence of ^{131}I-human albumin as a space marker. The membrane GPIIb-IIIa complex was examined by crossed immunoelectrophoresis (CIE) in the presence of rabbit anti-human platelet antiserum. The prolonged administration of PCR 4099 inhibited almost completely platelet aggregation induced by 0.5 to 10 µM ADP. Although the effect of ADP on aggregation was blocked at high concentrations, PCR 4099 did not modify ADP-induced shape change. Only the effects of low concentrations of thrombin (< 0.05 U/ml) were inhibited by PCR 4099 administration. The binding of ^{125}I-fibrinogen was reduced by 50 to 90 % when platelets were stimulated by 5 µM ADP or by 0.05 U/ml thrombin. PCR 4099 did not modify the pattern of immunoprecipitates as revealed by CIE. In particular the GPIIb-IIIa complex was not dissociated and its electrophoretic mobility was not changed. In conclusion, PCR 4099, which is more potent than ticlopidine in man, inhibits specifically the ADP aggregation pathway by interfering with the binding of fibrinogen to the GPIIb-IIIa complex in platelets having undergone shape change.

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INTERACTIONS BETWEEN PGE_2 AND INHIBITORS OF PLATELET AGGREGATION THAT ACT THROUGH cAMP. S.J. Gray and S. Heptinstall. Department of Medicine, University Hospital, Nottingham, NG7 2UH, U.K.

PGE_2 has a biphasic effect on platelet aggregation with low concentrations of the prostaglandin potentiating aggregation and high concentrations inhibiting it. In this investigation we have studied the interaction of PGE_2 with agents that inhibit platelet aggregation through an effect on cAMP. The agents chosen raise the level of cAMP in platelets by different mechanisms: PGI_2, PGD_2 and adenosine combine with specific surface-located receptors and stimulate adenylate cyclase (AC) via a guanine nucleotide-binding protein (GNBP), forskolin stimulates AC directly, and AH-P 719 and DN 9693 inhibit cAMP phosphodiesterase (PDE). ADP-induced platelet aggregation was measured in platelet-rich plasma and cAMP was measured in platelets labelled with ^{3}H-adenine.

PGE_2 alone potentiated platelet aggregation at concentrations from 10^{-8}-10^{-6}M and inhibited aggregation at 10^{-5}M. PGE_2 did not reduce cAMP levels at any concentration and increased cAMP levels at concentrations > 10^{-6}M, probably by stimulating AC.

PGI_2 (10^{-9}-10^{-8}M), PGD_2 (10^{-7}-$5x10^{-6}$M) and adenosine ($8x10^{-5}$-$2x10^{-4}$M) increased the level of cAMP in platelets and inhibited aggregation. These changes were reversed by low concentrations of PGE_2 (10^{-8}-10^{-6}M).

Forskolin ($5x10^{-6}$-$2.5x10^{-5}$M), AH-P 719 (10^{-7}-10^{-5}M) and DN 9693 ($5x10^{-6}$-10^{-5}M) increased the level of cAMP in platelets and inhibited aggregation. However, PGE_2 did not reverse the inhibitory effects of these particular agents. In contrast, PGE_2 potentiated the effects of the agents at all the concentrations of PGE_2 that were tested (10^{-8}-10^{-5}M).

The different results obtained with PGE_2 in combination with agents that act via surface-located receptors compared with agents that stimulate AC directly or act through PDE, suggest that PGE_2 may potentiate platelet aggregation by acting at a point between the platelet receptor and AC i.e. GNBP.

PGE_2 is one of the major prostaglandins synthesised by human microvascular endothelial cells and interstitial cells of the renal medulla. Since it reverses the inhibitory effects of some AC stimulators but adds to those of PDE inhibitors, the latter may have greater potential as anti-thrombotic agents in the micro-circulation and intra-renal circulation.